Expression of activation antigens CD69, HLA-DR, interleukin-2 receptor-alpha (IL-2Rα) and IL-2Rβ on T cells of human decidua at an early stage of pregnancy

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SUMMARY

T cells of human decidua at an early stage of pregnancy were examined by flow cytometry for the expression of the T-cell-activation antigens CD69, HLA-DR, interleukin-2 receptor-alpha (IL-2Rα) and IL-2Rβ. The decidua contained a small number of T cells and both $CD4^+$ and $CD8^+$ subsets expressed CD69, HLA-DR, IL-2Rα and IL-2Rβ antigens significantly whereas, in peripheral blood, only a small number of T cells expressed these activation antigens. These findings indicate that T cells in the decidua in the first trimester of pregnancy are regionally activated.

Since the decidualized endometrium is the site of fertilized ovum implantation and placental invasion, mechanisms for the maintenance of pregnancy may be elucidated by examining decidual tissue for immunocompetent cells.

Recent studies have suggested that maternal T cells play an important role in maintaining pregnancy in the mouse.[1–6] Wegmann proposed that immunostimulation, rather than suppression, is important in maintaining pregnancy. This concept, termed the 'immunotrophism hypothesis', is based on the report that a soluble T-lymphocyte factor [i.e. granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3)] enhances the proliferation of murine trophoblast cells *in vitro*. If the immunotrophism model is valid in humans, T cells in the decidua should be in an activated state, because these cytokines are not secreted from T cells in the resting state. Therefore, we examined whether human intradecidual T cells express the activation antigens, CD69, HLA-DR, interleukin-2 receptor-alpha (IL-2Rα) and IL-2Rβ by flow cytometric analysis.

Tissue samples were obtained from 12 women who had indicated elective abortions of normal pregnancy between the sixth and twelfth week of pregnancy under informed consent. Decidual tissue and peripheral blood samples were taken from each woman when the abortions were performed. The decidual tissue was macroscopically separated from chorionic villi, and then cut into small pieces and vigorously shaken for 1 min without enzymatic treatment. These samples were then filtered through a 32-μ nylon mesh, and decidual mononuclear cells were isolated by the Ficoll–Hypaque gradient sedimentation.

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Peripheral blood mononuclear cells (PBMC) were also isolated by the standard Ficoll–Hypaque method.

The following monoclonal antibodies (mAb), purchased from Becton Dickinson Immunocytometry System (San Jose, CA), were used: (1) CD3 (Leu-4), CD4 (Leu-3), CD8 (Leu-2), CD14 (Leu-M3), CD16 (Leu-11), CD20 (Leu-16) and CD56 (Leu-19); and (2) CD69 (Leu-23), HLA-DR (HLA-DR), CD25; IL-2Rα, p55(2A3) and CD45 (HLe-1). The first seven of these mAb were labelled with phycoerythrin (PE), and the last four and TU27 (IL-2Rβ, p75) mAb, which was kindly given by Dr Sugamura (Tohoku University, Japan), were labelled with fluorescein isothiocyanate (FITC).

The size of decidual lymphocytes ranged from that of resting peripheral blood lymphocytes to monocytes, and thus decidual macrophages were unable to be excluded with scatter gate. The lymphocyte population, identified by forward and size scatter gates, contained $86{\cdot}8 \pm 6{\cdot}6\%$ (mean ± SD, $n = 12$) lymphocytes and $6{\cdot}6 \pm 4{\cdot}1\%$ (mean ± SD, $n = 12$) macrophages, as analysed by two-colour flow cytometry with CD45–FITC and CD14–PE.

Flow cytometry was performed on a FACScan (Becton Dickinson). The number of cells counted was 50,000. Positive rates were expressed as percentages of positive cells in all lymphocytes (excluding monocytes/macrophages).

The percentage of $CD16^-$ $CD56^{bright}$ natural killer (NK) cells, which have been called endometrial granulocytes,[7–10] was $84{\cdot}8 \pm 5{\cdot}2\%$ of the decidual lymphocytes, whereas the percentage of $CD16^+$ NK cells was very low ($2{\cdot}7 \pm 1{\cdot}2\%$). The percentage of $CD3^+$ T cells was $12{\cdot}2 \pm 4{\cdot}6\%$, which was markedly lower than that in peripheral blood. These cell populations are similar to those described in previous studies.[8–10] The percentages of $CD4^{bright}$ cells and $CD8^{bright}$ cells were $5{\cdot}6 \pm 2{\cdot}1\%$ and $4{\cdot}7 \pm 1{\cdot}9\%$, respectively. The CD4/CD8 ratio was $1{\cdot}23 \pm 0{\cdot}42$.

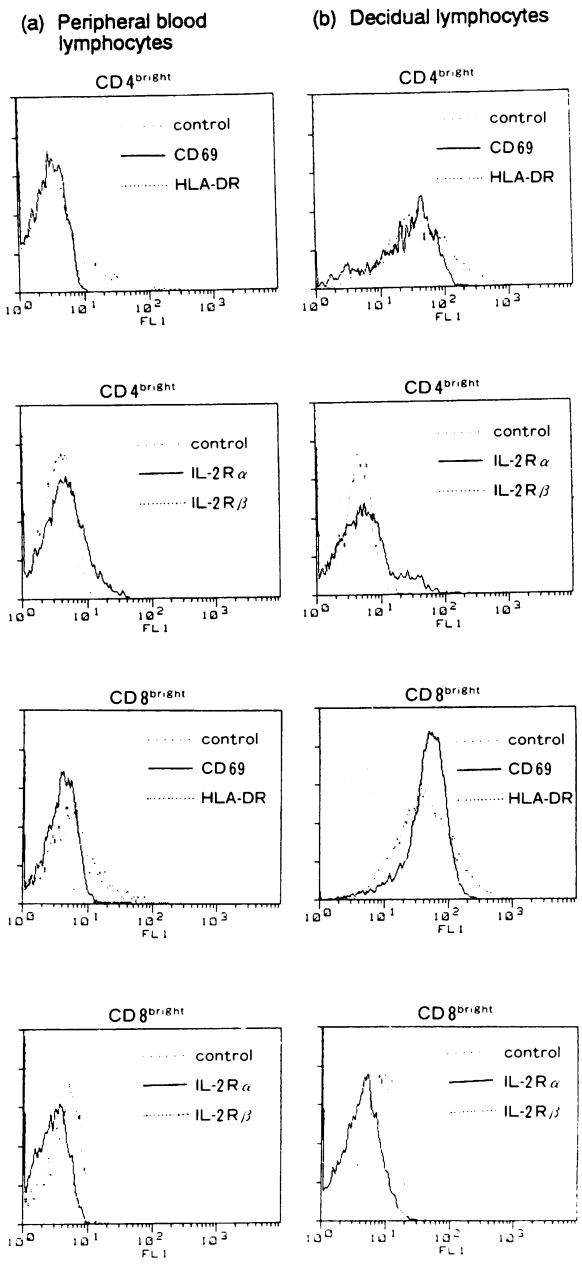

Figure 1. Expression of CD69, HLA-DR, IL-2Rα and IL-2Rβ on peripheral blood $CD4^{bright}$ cells, peripheral blood $CD8^{bright}$ cells, decidual $CD4^{bright}$ cells and decidual $CD8^{bright}$ cells. PBL and decidual lymphocytes were stained with FITC-conjugated anti-CD69, HLA-DR, IL-2Rα and IL-2Rβ and PE-conjugated anti-CD4 or CD8. Samples were analysed by FACScan and displayed as histograms. Since decidual macrophages were unable to be excluded with the scatter gate, we set the marker tightly on each subset, $CD4^{bright}$ and $CD8^{bright}$ cells.

The expression of the CD69 antigen (an early activation marker of T and NK cells)[11,12] by decidual $CD4^{bright}$ and $CD8^{bright}$ cells was 58·3 ± 12·7% and 73·3 ± 14·3%, respectively. On the other hand, peripheral blood $CD4^+$ and $CD8^+$ cells in early pregnant women express CD69 in only 0·9 ± 0·5% and 3·5 ± 0·7% of cells, respectively (Fig. 1, Table 1). CD69 antigen has been reported to appear shortly after stimulation via protein kinase C (PKC). Our finding indicates that the T cells in the decidua of pregnant women have already been stimulated via PKC.

Table 1. T-cell activation marker expression on decidual and peripheral blood T cells in the first trimester of pregnancy

	Decidual T cells ($n=12$)	Peripheral blood T cells ($n=12$)
CD4:CD69	58·3 ± 12·7†****	0·9 ± 0·5
HLA-DR	67·7 ± 14·8****	11·4 ± 4·6
IL-2Rα	26·9 ± 5·0*	20·5 ± 5·2
IL-2Rβ	8·6 ± 4·6***	1·5 ± 0·5
CD8:CD69	73·3 ± 14·3****	3·5 ± 0·7
HLA-DR	82·9 ± 13·5****	18·6 ± 8·0
IL-2Rα	6·2 ± 2·9**	2·5 ± 1·5
IL-2Rβ	17·7 ± 7·2*	10·6 ± 3·4

† % of $CD4^+$ or $CD8^+$ cells. All values are mean ± SD.

Statistical significances were noted between decidual T cells and peripheral blood T cells: * $P<0·05$; ** $P<0·01$; *** $P<0·001$; **** $P<0·0001$.

HLA-DR antigen (a relatively late activation T-cell marker) was detected in 11·4 ± 4·6% of $CD4^+$ cells and in 18·6 ± 8·0% of $CD8^+$ cells in peripheral blood lymphocytes of pregnant women (Table 1). In contrast, in the decidua, most T cells showed HLA-DR antigen expression (67·7 ± 14·8% of $CD4^+$ cells and 82·9 ± 13·5% of $CD8^+$ cells) (Table 1, Fig. 1). Thus, we show here that decidual T cells express an early activation antigen, CD69, and a late activation antigen, HLA-DR. In relation to this, it is interesting to know to what extent IL-2 receptors are expressed. It has been reported that a proportion of resting $CD4^+$ T cells express IL-2Rα and resting $CD8^+$ T cells express IL-2Rβ slightly.[13,14] However, decidual $CD4^+$ cells express not only IL-2Rα but also IL-2Rβ. Decidual $CD8^+$ T cells express not only IL-2Rβ but also IL-2Rα. The level of antigen expression was slight but significant (Fig. 1, Table 1). These results were not caused by contamination with either $CD56^{bright}$ cells, which possess IL-2Rα and IL-2Rβ,[10–15] or $CD16^+$ cells and monocytes which possess IL-2Rβ,[13,14,16] because we set the marker tightly on each subset, $CD4^{bright}$ and $CD8^{bright}$ cells. Bulmer and Johnson reported that, using an immunohistochemical method, decidual lymphocytes did not possess IL-2Rα.[17] In our recent flow cytometric study, we observed the expression of both IL-2Rα and IL-2Rβ by $CD16^-$ $CD56^{bright}$ NK cells, the major population of decidual lymphocytes.[10] The discrepancy between our study and other earlier studies is probably attributable to the higher sensitivity of flow cytometry compared with immunohistochemical methods.

In this study, both $CD4^+$ and $CD8^+$ T cells in the decidua expressed the activation antigens CD69, HLA-DR, IL-2Rα and IL-2Rβ. It has been reported that anti-CD8 treatment of pregnant mice reduces placental proliferation and phagocytosis, and that anti-CD4 antibody treatment affects placental proliferation.[5] Thus, it would appear that activated CD4- and CD8-positive cells may be involved in placental cell proliferation and function.

Another important question concerns the mechanism of T-cell activation in the decidua. Since trophoblast produces an IL-2-like molecule and expresses IL-2 messenger RNA (mRNA) at

an early stage of pregnancy,[18,19] it is possible that this factor may activate T cells. However, it is also possible that other antigen-presenting cells in the decidua are involved in T-cell activation.[20] Immunosuppressive factors, such as transforming growth factor-beta (TGF-β) and prostaglandin E2, are known to be distributed regionally in the decidua.[21,22] These factors could influence the expression of IL-2Rα and IL-2Rβ, because expression of these activation markers was slight compared with CD69 and HLA-DR. In the present study, T cells appeared phenotypically to be in an activated state, even in the presence of immunosuppressive factors. Pregnancy thus seems to be maintained by the regulation of immunosuppressive and immunopotentiating mechanisms.

The next stage of investigation should address the question of whether activated T cells in the human decidua secrete cytokines (e.g., IL-3 and GM-CSF) in the area of fertilized egg implantation.

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